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Anti-diabetic and renal protective effect of the fruit juice of *Citrus X Paradisi* on alloxan induced diabetic male albino wistar rats

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ABSTRACT

Diabetes has become a global health concern, its management lies hugely on expensive medical care and monitoring; thus the need to investigate possible alternatives to reduce treatment cost. This study aims to assess the anti-diabetic, lipid lowering and renal protective effect of *Citrus X paradisi* on alloxan induced diabetic male albino Wistar rats. Twenty-five (25) male albino Wistar rats, weighing 140g-230 g were obtained for the study. The animals were divided into five (5) groups of five (5) animals each according to their weights. Diabetes was induced by intravenous injection of 150mg/kg of 5% alloxan monohydrate in citrate buffer, pH 4.0. Group A received (0.1ml) 250mg/kg of the grape juice extract; Group B received 0.4ml, 500mg/kg, Group C received 0.7ml 1000mg/kg; Group D (Group D positive control) received 0.9ml (5ml/kg) of glibenclamide; and Group E (diabetic negative control) received only water. Blood glucose was estimated using Accu-Chek glucose meter; triglyceride, urea and creatinine were estimated using respective colorimetric methods. Animals in Group A recorded significantly lower mean blood glucose level at 4 and 8 hours compared to the 0 hour specimen ($P < 0.001$). Group C animals recorded significantly reduced blood glucose level at 8 hours compared to the fasting sample (0 hour specimen) ($P < 0.05$). In the sub-acute study, animals in group A and C recorded significant decreases in their mean blood glucose level at the 8th day for group A and 4th day for group C compared to 0 hour specimen ($P < 0.001$). A significant reduction in the levels of triglyceride, urea and creatinine of animals in group A, B, C, and D were observed compared to the negative control group. Results obtained from this study show that the juice extract of *Citrus X paradisi* could have effect in managing diabetes and preventing its complications.

Keywords: Diabetes, triglyceride, creatinine, urea, anti-hyperglycemic, alloxan, glucose

INTRODUCTION

The need for alternative, inexpensive and readily available remedies for the treatment of diabetes mellitus has provoked strong research works around the world. The use of herbal products and plant fruits has been experimented and remarkable successes recorded. The prevalence of diabetes of all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 and the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [1]. Alternative remedies such as use of plant extracts and fruits are used worldwide to

ameliorate the complications of diabetes [2]. Several medicinal plants have been shown to have hypoglycemic properties [3] [4].

Grapefruit seed extract has specifically shown anti-oxidant and anti-hyperglycemic effects. The fruit juice has been shown to have similar effect on domestic consumers. Grapefruit, also known as, *Citrus paradisi* was first described in 1750 by Griffith Hughes who called it the forbidden fruit of Barbados [5]. All the parts of the fruit, namely : the peel, the seed and the juice have been shown to have several medicinal uses such as anti-oxidants, anticancer, anti-diabetic, anti-hypertensive and numerous other effects [6].

Diabetes mellitus is a group of diseases marked by high levels of blood glucose also called blood sugar, resulting from defects in insulin production, insulin action or both [7]. The WHO defines diabetes as a chronic disease that occurs when the pancreas does not produce enough insulin or the body cannot effectively use the insulin it produces. There are four types of diabetes mellitus: Type I diabetes mellitus (also called Insulin-Dependent Diabetes Mellitus (IDDM), type II Diabetes Mellitus (formerly called Non-Insulin dependent Diabetes Mellitus (NIDDM), gestational diabetes mellitus and malnutrition or genetic related diabetes mellitus [8].

Alloxan, a drug derived from uric acid induces diabetes in rats by selectively destroying the Langerhans islet cells [9]. It has successfully been used to induce diabetes in experimental animals through the subcutaneous or intraperitoneal means. Numerous studies demonstrate that a variety of plant extracts effectively lowered the glucose level in alloxan-induced diabetic animals [10] [11] [12] [13]. Thus this study is aimed at evaluating the anti-hyperglycemic; lipid lowering and renal protective effect of grape fruit juice extract on alloxan induced diabetes male albino Wistar rats.

MATERIALS AND METHODS

3.1 Animal husbandry

Twenty-five (25) male albino Wistar rats, weighing 140g-230 g were obtained from the animal house of the University of Nigeria, Enugu Campus (UNEC). They were housed under standard conditions of temperature ($28\pm 3^{\circ}\text{C}$) and a 12-hour light/12-hour dark cycle. The animals were housed in groups and were provided with water and standard pellets (Guineafeed) ad libitum. The period of acclimatization was 2 weeks.

3.2 Fruit Collection and Identification

The fruits of *Citrus x paradisi* (white variety) used for this work were harvested from the parent tree in Ugbo-Okpala, Awgu Local Government Area, Enugu state in the month of June. A specimen was identified by a taxonomist, of the department of Botany, University of Nigeria, Nsukka.

3.3 Juice Extraction

After harvesting the fruits, they were peeled fresh until the pulps were visible. The pulps were cut into tiny pieces to fit into the receptacle of the juice extractor. The seeds were removed while cutting the pieces. 1500ml of the extracted juice was filtered through a muslin bag and then later using the Whatman filter paper to ensure the collection of the pure juice. The filtrate was frozen under standard condition to avoid fermentation and possible deterioration of the chemical components of the juice extract. After forty-eight (48) hours of refrigeration the pure extract was obtained by collecting the thawing liquid drop by drop until about 500ml was collected. The rest were discarded.

3.4 Determination of Extractive Value

The concentration of the pure extract was determined by evaporating 0.1ml of the extract in an evaporating dish of known weight in an oven (Gallen Kamp, UK) to dryness at 60°C . The dish containing the residue was allowed to cool and weighed. The weight of the residue was obtained by subtracting the weight of the empty dish from the weight of the dish and residue. The above process was repeated twice and an average weight taken. The extractive value was 250mg/ml.

3.5 Experimental Design and Conduct

The animals were divided into five (5) groups of five (5) animals each according to their weights. All the animals were made to fast overnight but had free access to water. The baseline plasma glucose of the five (5) groups of twenty five (25) rats were determined prior to the induction of diabetes by subcutaneous injection of 150mg/kg of

5% of alloxan in citrate phosphate buffer; pH4.0. They were left for 24 hours after which their blood glucose levels were determined.

Group A received (0.1ml) 250mg/kg of the grape juice extract; Group B received 0.4ml, 500mg/kg, Group C received 0.7ml 1000mg/kg; Group D (diabetic positive control) received 0.9ml (5ml/kg) of glibenclamide; and Group E (diabetic negative control) received only water.

3.5.1 Acute Study

All the rats were made to fast overnight till the next morning and their fasting blood glucose level determined (0 hour) before which the extracts were administered via oral route. Then blood glucose levels were measured at intervals of 2 hours, 4 hours, 8 hours and 24 hours.

3.5.2 Sub-acute Study

This second phase began with an oral administration of the extracts every morning for 8 days. The fasting blood glucose levels of all the animals were determined before the extract administration (0 day). Blood glucose levels were determined again on day 4 and finally on day 8. Blood was then collected through puncture of the retroorbital vein of the eye for assay of urea, creatinine and triglyceride.

Determination of Blood Glucose Levels

The blood glucose level of each animal was measured by the use of a blood glucose meter, Accu-Chek. Blood used for the test was obtained by pricking the distal end of the tail and placing the drop of blood on the test strip.

Determination of serum triglyceride concentration

Serum triglyceride concentration was determined using enzymatic colorimetric method. The kit was obtained from Randox international UK.

Determination of serum Urea concentration

Serum urea concentration was determined using the diacetyl monoxime method with protein precipitation (Nelson, 1951).

Determination of serum creatinine concentration

Serum creatinine concentration was determined using the Jaffe Reaction (Fabing, 1971)

Statistical analysis

Data was analyzed using SPSS software version 18. All data were expressed as mean \pm SEM. Levels of significance was determined by the student t-test while main effects of treatment (group) were determined by the one way analysis of variance (ANOVA) followed by the Tukey's Post-HOC multiple comparison test. $P < 0.05$ or $P < 0.001$ were considered significant.

RESULTS

Anti-hyperglycemic and renal protective effect of grape juice extract was assessed in alloxan induced diabetic male albino Wister rats. Table 1 shows the effect of grape fruit (*Citrus x paradisi*) juice extract on the blood glucose level of alloxan induced diabetic male albino Wister rats compared to their respective 0 hour blood glucose level (acute study). Animals in Group A that received 250mg/kg body weight of the grape juice extract recorded significantly lower mean blood glucose level at 4 and 8 hours compared to the 0 hour specimen ($P < 0.001$). Group C animals that received 1000mg/kg of the grape juice extract recorded significantly reduced blood glucose level at 8 hours compared to the fasting sample (0 hour specimen) ($P < 0.05$). The positive control animals (group D) that received 5mg/kg of glibenclamide showed a significant decrease in mean blood glucose level at 4 hours compared to the fasting specimen ($P < 0.05$). No significant variation was observed in the blood glucose level of group B animals compared to their fasting specimen ($P > 0.05$). while the group E animals (diabetic negative controls) that received only water and feed showed increasing blood glucose level.

The effect of grape fruit (*Citrus x paradisi*) juice extract on the blood glucose level of alloxan induced diabetic male albino Wister rats (sub-acute study) are shown in table 2. Here animals in group A that received 250mg/kg of the juice extract recorded significant decrease in their mean blood glucose level at the 8th day compared to the day before the extract was feed (0 day) ($P < 0.001$). Group C animals that received 1000mg/kg of the juice extract

recorded significantly lower mean blood glucose level at the day 4 and 8 compared to 0 day blood glucose level ($P < 0.01$), while the positive control animals that received 5mg/kg glibenclamide showed a significant reduction in their blood glucose level at the 8th day compared to the day before the extract was feed ($P < 0.001$). Animals in group E (diabetic negative controls) that received only water and feed showed increasing blood glucose level.

Comparison of serum triglyceride, urea and creatinine levels of the various groups of grape juice feed extract with the positive and Negative controls are represented in table 3. Animals that received 500mg/kg of the juice extract (group B) and those that received 1000mg/kg of the extract (group C) showed a significant decrease in their serum triglyceride ($P < 0.001$) and creatinine level ($P < 0.05$) compared to the positive (glibenclamide) control group. A significant reduction in the levels of triglyceride and urea of animals in group A, B, C, and D were observed compared to the negative control group (those animals that received only water and feed) ($P < 0.001$). Serum creatinine level was significantly decreased in groups A and D ($P < 0.001$) and in B ($P < 0.05$) compared to the negative control.

TABLE 1 Effect of grape juice extract on the blood glucose levels (mg/dl) of the various groups in comparison with their respective fasting blood glucose level (0 hour) (Acute Study)

Groups Time (Hour)	A (250mg/kg extract)	B (500mg/kg extract)	C (1000mg/kg extract)	D (5mg/kg Glibenclamide)	E (water and feed)
0	414.75±29.81	191.75±13.12	286.75±31.07	249.50±32.80	199.75±7.81
2	354.33±26.96	129.00±16.29	241.00±0.8	141.83±23.63	213.67±23.54
4	174.80±31.75**	184.80±17.71	220.20±32.83	95.60±12.02*	238.80±24.87
8	65.80±6.03**	268.00±36.67	132.00±18.12*	135.80±8.24	288.60±24.06
24	372.80±19.96	386.20±31.60	360.20±35.55	393.80±51.58	279.40±32.02

Values given as Mean±SEM

* $P < 0.005$ with respect to 0hr specimen (FBS)

** $P < 0.001$ with respect to 0hr specimen (FBS)

TABLE 2 Comparison of the Blood Glucose (mg/dl) of the Groups with the Glucose Concentration of the Animals at the Beginning of the Experiment (0 day) (Sub acute study)

Groups Time (Days)	A (250mg/kg extract)	B (500mg/kg extract)	C (1000mg/kg extract)	D (5mg/kg Glibenclamide)	E (water and feed)
0	418.00±10.17	408.20±23.97	411.60±26.99	488.60±26.99	175.80±9.37
4	395.80±8.84	386.00±37.088	259.20±53.27*	437.40±35.75	257.60±24.54
8	188.20±4.81**	512.20±21.08	237.60±20.58*	191.80±27.29**	243.40±16.24

Values given as Mean±SEM

* $P < 0.05$ with respect to glucose at the beginning of experiment

** $P < 0.001$ with respect to glucose at the start of experiment

Table 3 Comparison of Serum Triglyceride, Urea and Creatinine Levels of the groups with the Positive and Negative Controls

Groups	TG (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
A(250mg/kg extract)	94.60±5.52 ^a	43.20±5.98 ^b	1.16±0.09 ^a
B(500mg/kg extract)	53.60±3.34 ^{a,c}	51.40±5.56 ^b	1.56±0.12 ^{b,d}
C(1000mg/kg extract)	147.00±3.13 ^{a,c}	51.00±4.58 ^b	1.84±0.24 ^c
D (5mg/kg extract)	87.80±2.20 ^a	35.20±5.02 ^a	1.10±0.10 ^a
E (water and feed)	192.00±4.51	77.60±3.44	2.0±0.44

Values expressed as Mean±SEM

^a= $P < 0.001$, ^b=0.05 with respect to negative control

^c= $P < 0.001$, ^d=0.05 with respect to positive control

FIG 1 A curve of the mean fasting blood glucose (mg/dl) levels showing the acute glycemic changes over 24 hours

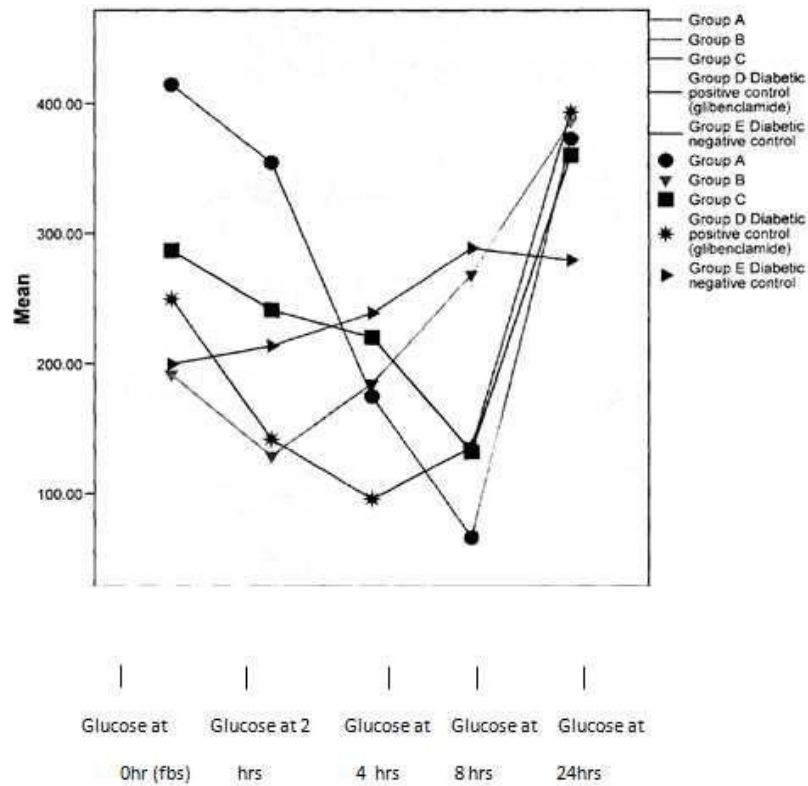
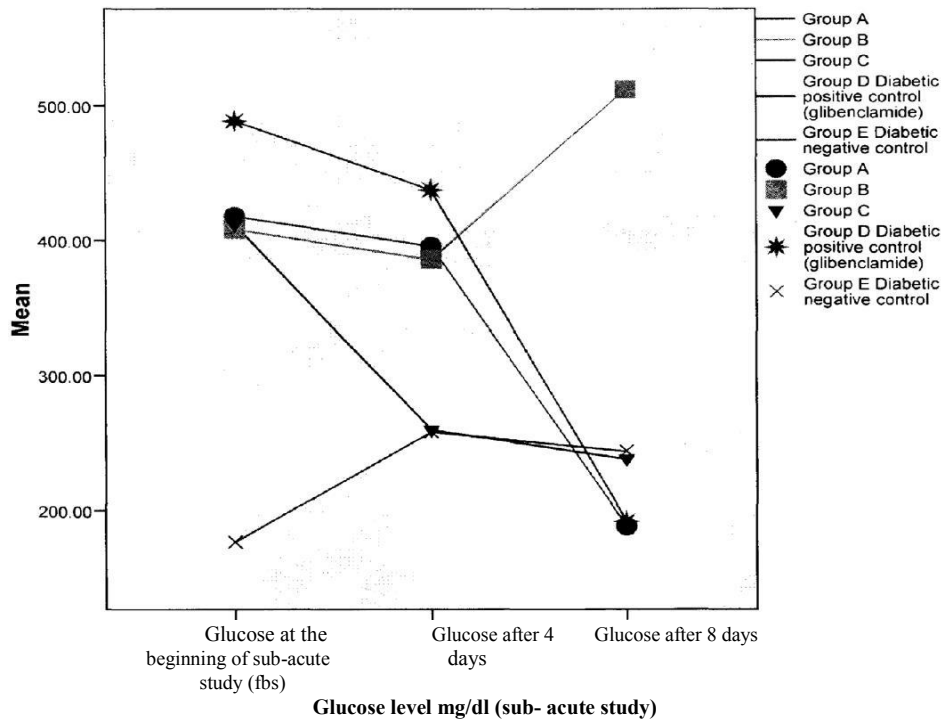


FIG 2 A curve of the mean fasting blood glucose (mg/dl) levels showing the sub acute glycemic changes over 8 days



DISCUSSION

The fruit juice of *Citrus x paradisi* has been variously used in the treatment of several health conditions including diabetes. Diabetes is a metabolic disorder which can be considered as a major cause of high economic loss which can in turn impede the development of nations [14]. Moreover, uncontrolled diabetes leads to many chronic conditions such as blindness, heart failure and renal failure.

The results obtained from this study show that the fruit juice of *Citrus x paradisi* has anti-diabetic effect and also lowered the serum triglyceride, urea and creatinine in alloxan-induced diabetic rats used. It significantly lowered blood glucose in animals that received 250mg/kg of the extract at the 4 and 8 hours compared to the fasting level. Equally, sub-acute effect on the rats was observed as animals that received the lowest dose of the extract (250 mg/kg) showed statistically significant decrease in their blood sugar at the 8 day compared to the 0 day. This reduction at both the acute and sub-acute levels of blood glucose shown by the extract approximated the effect of glibenclamide, a sulfanylurea hypoglycemic agent used for the positive control.

The fruit juice of *Citrus x paradisi* has been shown to contain several phytochemicals such as polyphenolic compounds, resveratrol, flavonoids, flavon-3-ols, myricetin, peonidin, flavonols, quercetin, tannins, anthocyanins, kaempferol, cyanidin, ellagic acid and proanthocyanidins [15]. The anti-diabetic effect observed may be attributable to a single or combined effect of these phytochemicals especially proanthocyanidin which is believed to exert this effect by its antioxidant activity [16]. Resveratrol, another important phytochemical could also be responsible for the observed hypoglycemic effect as it has been shown to improve insulin resistance in diabetic rats. The glucose lowering effect of the extract approximated that of glibenclamide. So, it could be suggested that the mechanism of glycemic control may be similar to that of glibenclamide which acts by stimulating insulin release and the inhibition of glucagon secretion [17]. Stimulation of residual pancreatic beta cells function might also account for the hypoglycemic effect [18].

Goldberg (2001) found that abnormalities in insulin action are associated with lipid abnormality. This was observed in the animals after diabetes induction as indicated by the high serum triglycerides found in the negative control compared to the extract-treated groups. The lipid-lowering effect observed in the extract-treated groups compared to the negative control agrees with the work of Castilla *et al*, (2006) [19] who observed the effect to be due to quercetin, a grapefruit phytochemical. Also, the significantly lowered triglyceride observed in groups A-D compared to the negative control may be due to provinol, a flavonoid phytochemical present in grape juice [20]. Furthermore, the serum creatinine of the extract-treated group A decreased significantly when compared to the negative control. A similar decrease was also observed in the positive control groups that received glibenclamide.

The decreased serum creatinine levels suggest the potential of the extract in reducing diabetic nephropathy, a property similar to glibenclamide activity [8]. Research has long shown that diabetic patients are at an increased risk of developing specific complications including nephropathy, retinopathy, neuropathy and atherosclerosis. The proanthocyanidins of the grapefruit juice may have produced a renal-protective effect (of lowering serum creatinine to normal) in the rats as this is in consonance with the work of Fuji *et al* (2006) [21] who showed that proanthocyanidins of the grapefruit showed the strongest protective effect against high-glucose induced oxidative stress on cultured kidney cell. In addition to the observed serum creatinine-lowering effect of the extract on the rats, serum urea equally significantly reduced compared to the negative control. This may be similarly due to the renal protective effect of the proanthocyanidin contained in the extract.

CONCLUSION

This study showed that the juice extract of *Citrus x paradisi* (white variety) exhibited considerable anti-diabetic and hypolipidemic effect on alloxan-induced diabetic rats. It equally improved the kidney function of the rats. The results obtained, together with the other enormous research works on the health benefits of the grape juice may justify the widespread domestic use of the juice in handling several health conditions including diabetes mellitus. The majority of the work on grapefruit had been with the red variety obtained in the Americas. Interestingly, the white variety obtained in the Nigerian tropical environment showed similar effects.

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